



Original Article

Analyzing the Relationship between Obstetric Characteristics with Antenatal Depression

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ABSTRACT

The current study investigated the impact of growth hormone (GH) and polymorphism of calpain genes (*CAPN1*) on the changes in live weight and qualitative and quantitative indicators of meat productivity of young Kazakh White-headed cattle. *CAPN1* polymorphism is represented by two alleles, C and G, with a frequency of 0.12 and 0.88, three genotypes, CC, CG, GG, with a frequency of 0.06, 0.81, and 0.13, respectively. *GH* polymorphism - V and L alleles, with a frequency of 0.40 and 0.60, and VV, LV, and LL genotypes with a frequency of 0.31, 0.51, and 0.18, respectively. The *CAPN1^C* and *GH^V* alleles are preferred for improving the quantity and quality of meat productivity of the Kazakh white-headed cattle. Animals with the *CAPN1^{CC}* and *GH^{VV}* genotypes had a 6.9% higher body weight at 12 months of age ($P < 0.05$) compared to those with the *CAPN1^{GG}* and *GH^{LL}* genotypes. The highest pre-slaughter, slaughter, and carcass weights were obtained from carcasses of *CAPN1^{CC}* and *GH^{VV}* homozygous bulls. Their superiority over the *CAPN1^{GG}* and *GH^{LL}* genotypes was 19.3; 16.2 and 16.4 kg; 19.8; 19.1 and 16.1 kg, respectively. The difference in favor of these genotypes in terms of the meat ratio by 4.1% and 2.0% was provided by a 9.1% greater meat mass and 1.4% and 2.0% slaughter yield. The protein and fat content in the longissimus muscle of the CC and VV genotypes carcasses was higher by 0.28-2.13%. *CAPN1^{CC}* and *GH^{VV}* animals had a higher concentration of unsaturated fatty acids in their muscle lipids than *CAPN1^{CG}*, *CAPN1^{GG}*, and *GH^{LV}*, *GH^{LL}* genotypes. The best unsaturated to saturated fatty acids ratio and the ratio of arachidonic (C20:4) and linoleic (C18: 2) acids indicate the advantage in beef's dietary properties obtained from beef *CAPN1^{CC}* and *GH^{VV}* animals.

GRAPHICAL ABSTRACT

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Introduction

The use of genes related to product features to evaluate animal genotypes is a straightforward, in-demand, and accessible strategy for predicting the productive attributes of beef cattle at an early age [1-3]. In recent years, beef cattle breeding in Russia has expanded significantly due to implementing the sectoral program "Development of beef cattle breeding in Russia for 2013-2020" [4-6]. During this period, the volume of beef cattle production increased 4.1 times and is 20% of the total domestic beef production. In 2020, the livestock of specialized beef cattle was about 3.0 million. According to the Ministry of Agriculture of the Russian Federation, currently, about 60.0% of the total beef cattle livestock is bred in the Republic of Kalmykia - 8.7%, Orenburg region - 7.7%, Chelyabinsk region - 6.6%, Stavropol Territory - 5, 9%, Rostov region - 4.5%, and the Republic of Bashkortostan - 3.9% [7].

The Kazakh white-headed breed is the first early maturing meat cattle bred in Russia [8-10]. The breed has been formed in a sharply continental climate based on aboriginal Kazakh and Kalmyk breeds using the gene pool of the Hereford breed from 1930 to 1950 [11, 12]. Currently, it is widespread in the North Caucasus, the Urals, the Volga region, Western and Eastern Siberia [13].

The improvement of the productive qualities of beef cattle in general, and the Kazakh white-headed breed, in particular, at the present stage involves the use of molecular DNA markers along with the methods of classical breeding because genotyping allows one to assess the genotype of animals and determine their breeding value at the earliest age [14-16]. The most promising genetic markers for beef cattle breeding are myostatin - *MSTN* [17, 18], calpain - *CAPN*, calpastatin - *CAST* [14, 15], leptin - *LEP* [21, 22], retinoic acid receptor - *RORC-C* [23], and stearoyl-CoA desaturase - *SCD* [24, 25].

One of the lines of development of specialized beef cattle breeding is to obtain high-quality, so-called "marbled" beef by using genetic testing for marker genes [26]. Here, the calpain gene (*CAPN1*) deserves attention, as it controls the function of weakening the bundles of muscle fibers [27, 28]. Two SNPs, *CAPN1_316* (BTA29; rs17872000) and

CAPN1_4751 (BTA29; rs17872050), were found in *CAPN1*. *CAPN1_316* and *CAPN1_4751* were found to have two alleles each: C and G, and C and T, respectively. It was found that meat in homozygous CC in *CAPN1_316* animals is more tender [29-31].

The growth hormone (GH) gene plays an essential role in carbohydrate-fat metabolism [32-35]. In different breeds of cattle, several SNPs in the GH gene have been described, most of which are located in introns, some in the regulatory sequence, and only one in the fifth exon at position 2141. The substitution of C for G leads to a change in the protein's amino acid sequence; there is a leucine for valine replacement (Leu → Val). This SNP is associated with increased live weight and fat deposition in muscle tissue, which is a vital beef quality indicator of marbling [36-39].

Despite the importance of the Kazakh White-headed cattle for breeding in many regions of Russia, the polymorphism of the *GH* and *CAPN1* genes and their effect on productive properties have not been sufficiently studied, which served as a rationale for this study. This research study aimed to investigate the effect of GH and *CAPN1* genotypes on the meat production of Kazakh White-headed bulls in terms of quality and quantity.

Materials and Methods

The studies were conducted in the immunogenetics and DNA technologies laboratory of the All-Russian Research Institute of Sheep and Goat Breeding. The research object was the Kazakh White-headed bulls (n=93), Gigant agricultural production cooperative, Stavropol Territory, Blagodarnensky district. According to the DIAAtomtmDNAPrep protocol (Isogen, Moscow), DNA was isolated from blood samples of experimental animals using the DIAAtomtmDNAPrep kit (Isogen, Moscow) according to the manufacturer's protocol.

Feeding and keeping the experimental bulls were the same for all genotypes and were normalized by the rearing periods per the in-house norms. The changes in the live weight of the bulls were determined by weighing at 240 and 365 days. The difference in values in the recorded periods was used to calculate the average daily gain in live

weight. To study meat productivity and biochemical composition of meat, animals of each genotype were slaughtered according to V.I. Lenin All-Union Academy of Agricultural Sciences according to GOST R 54315-2012 [40].

The chemical composition of the longest Dorsi muscle (L. Dorsi) (amount of moisture, protein, fat, calorific value) was studied according to methods approved by interstate standards (GOST

"Meat and Meat Products," 2014, 2016, 2018) [41].

The fatty acid composition of the lipids of the longest Dorsi muscle was studied by gas-liquid chromatography on a Chromatek-Crystal 5000 (EZD-1 No. 800080, PID-1 No. 800698, with a Buchi rotary evaporator). Chromatek Analytic software package was used for data collection and processing. The following were used as integral indicators of the lipid metabolic intensity [37]:

$$\text{Lipid Saturation Index (LSI)} = \frac{\Sigma \text{ of saturated fatty acids, \%}}{\Sigma \text{ of unsaturated fatty acids, \%}} \quad (1)$$

$$\text{Metabolic Efficiency Ratio (MER)} = C_{20:4}/C_{18:2} \quad (2)$$

The obtained values were biometrically processed using the Microsoft Office and BIOSTAT software package. Based on the mean values and standard errors, the reliability of the difference between the mean values obtained by calculating and measuring the studied parameters using the student's t-test was calculated.

Results and Discussion

The genotyping results showed that *CAPN1* polymorphism in Kazakh White-headed bulls is represented by two alleles, C and G, with a frequency of 0.12 and 0.88, three genotypes, CC, CG, GG, with a frequency of 0.06, 0.81, and 0.13,

respectively. *GH* polymorphism - V and L alleles, with a frequency of 0.40 and 0.60, and *VV*, *LV*, and *LL* genotypes with a frequency of 0.31, 0.51, and 0.13, respectively.

The revealed polymorphism made it possible to group the bulls by the established genotypes and study the changes in their live weight and, at the age of 12 months, their chemical and fatty acid composition of the longest muscle (*L. Dorsi*).

Comparing the live weight of bulls of different genotypes in different periods showed an advantage of C- and V-allele carriers in *CAPN* and *GH*, respectively, both at birth and the age of 365 days (Table 1).

Table 1: Changes in live weight of the bulls of different genotypes by *GH* and *CAPN1*

Gene	Genotype	Live weight, kg		Average daily gain, g
		at birth	365 days	
CAPN1	CC	28.1±0.21	374.2±5.1	948.2±9.21
	CG	27.9±0.18	368.9±3.6	934.2±7.26
	GG	27.6±0.16	349.9±4.1	883.0±8.53
GH	VV	28.3±0.11	382.7±4.7	970.9±6.54
	LV	27.9±0.18	373.8±4.2	947.6±9.20
	LL	27.6±0.12	357.9±5.2	904.9±7.17

However, significant differences were observed at the age of 12 months between the bulls with the homozygous alleles and those animals without the indicated alleles in their genotype. The difference between *CAPN1^{CC}* and *CAPN1^{GG}*, *GH^{VV}* and *GH^{LL}*, respectively, was 24.3 kg or 6.9% ($P < 0.05$) and 24.8 kg or 6.9% ($P < 0.05$). A similar superiority was found in the average daily gain in live weight

from birth to one year of age, which amounted to 7.4% ($P < 0.05$) and 7.35% ($P < 0.01$).

A comparative analysis of the slaughter indicators of bulls of different genotypes revealed the highest pre-slaughter, slaughter weight, and carcass weight in the homozygous *CAPN1^{CC}* and *GH^{VV}* genotypes *CAPN1* and *GH* genes. Their superiority over *CAPN1^{GG}* and *GH^{LL}* genotypes were 19.3; 16.2 and 16.4 kg, and 19.8; 19.1 and 16.1 kg,

respectively ($P < 0.05$; 0.01). Higher meet mass and slaughter yield by 9.0% and 9.1% ($P < 0.05$) and by 1.4 and 2.0%, respectively, provided these genotypes with a prevalence of the meat ratio by 4.1 and 2.0% (Table 2).

Table 2: Slaughter indicators of carcasses of Kazakh white-headed bulls of different genotypes for *CAPN1* and *GH* genes (12 months)

Indicators	Gen/genotype					
	CAPN1			GH		
	CC	CG	GG	VV	LV	LL
Pre-slaughter live weight, kg	380.2±3.02	371.3±2.23	362.3±2.06	379.8±1.98	369.6±2.45	359.8±3.12
Slaughter weight, kg	219.6±3.11	209.9±1.96	198.6±2.06	230.1±1.69	220.6±1.98	210.2±1.56
Carcass weight, kg	209.6±2.33	199.8±2.06	193.2±2.63	216.1±2.84	209.6±2.48	200.3±1.26
Meat weight after deboning, kg	164.6±1.87	156.7±1.53	150.9±1.45	170.1±2.18	162.9±1.86	155.9±0.54
Bone weight, kg	40.3±0.32	39.1±0.18	38.5±0.19	41.6±0.18	40.2±0.12	38.9±0.18
Internal fat weight, kg	10.3±0.23	9.6±0.32	10.5±0.12	13.1±0.31	11.9±0.22	10.1±0.39
Cartilage and tendon weight, kg	5.9±0.18	4.9±0.19	5.0±0.17	3.2±0.22	4.0±0.19	4.0±0.18
Slaughter yield, %	58.3	57.1	56.9	59.6	58.9	57.6
Carcass yield, %	55.6	54.5	54.0	56.1	55.7	54.8
Meat yield, %	78.1	78.0	77.6	79.1	78.7	78.4
Bone yield, %	19.1	19.4	19.8	19.5	19.4	19.6
Meat factor	4.08	4.01	3.92	4.09	4.05	4.01

Comparison of the chemical analysis indicators of the longest dorsal muscle revealed the superiority of the *CAPN1^{CC}* and *GH^{VV}* genotypes in terms of protein and fat content over other genotypes in the range of 0.28-2.13% (Table 3).

Table 3: ¹H NMR spectroscopy interpretation of the compound C1

Indicator	Genotype					
	CAPN1			GH		
	CC	GC	GG	VV	LV	LL
Total moisture, %	71.69±0.53*	75.12±0.41	74.63±0.23	75.25±0.35	74.29±0.32	75.75±0.29
Dry matter, %	25.96±0.22*	24.91±0.21*	25.63±0.31	26.04±0.27	25.89±0.18**	24.96±0.24
Protein, %	23.02±0.29**	22.04±0.32	20.89±0.43	29.85±0.36**	22.08±0.20**	22.10±0.16
Fat, %	2.93±0.03*	2.51±0.04	2.42±0.04	3.01±0.05*	2.82±0.05	2.59±0.03*
Ash content, %	1.22±0.02	1.19±0.02	1.21±0.02*	1.24±0.02	1.22±0.02	1.09±0.02*
Calorific value, kcal	1201.6±8.6**	1109.5±3.7*	1111.6±3.2	1233.4±7.2*	1183.7±4.9	1119.8±8.8

Note: * $P < 0.05$, ** $P < 0.01$ in comparison with *CAPN1* – CC-GG; *GH* – VV- LL

Chromatographic analysis of the lipids of the longest dorsal muscle in experimental bulls identified 34 fatty acids, of which 14 were saturated fatty acids, nine monounsaturated, and 11 polyunsaturated or unsaturated fatty acids. The content of individual fatty acids was so insignificant and did not differ between genotypes

that it was justified to be presented as a total (Table 4).

Comparison of the content of individual fatty acids in muscle tissue did not reveal a significant difference between animals of different genotypes. At the same time, there was a tendency of the superiority of bulls with *CAPN1^{CC}* and *GH^{VV}*

genotypes in the sum of monounsaturated fatty acids C_{14:1}-C_{17:1} and polyunsaturated fatty acids C_{18:2} ω₆ and C_{20:4} over animals with *CAPN1^{CC}*, *CAPN1^{GG}*, and *GH^{LV}*, *GH^{LL}* genotypes. A more pronounced difference between the indicated genotypes was observed in the total unsaturated fatty acids. In animals of *CAPN1^{CC}* and *GH^{VV}* genotypes, it was 50.34% and 50.77%, while in *CAPN1^{CG}*, *CAPN1^{GG}* и *GH^{LV}*, *GH^{LL}* genotypes it was 49.73%; 49.89% and 48.82% and 49.10% respectively. The higher content of unsaturated

fatty acids and their excess over the level of unsaturated fatty acids also determined lower lipid metabolic index (LSI) in animals of *CAPN1^{CC}* and *GH^{VV}* genotypes - 0.97 and 0.97, compared with 1.00 and 1.04 in *CAPN1^{GG}* and *GH^{LL}* genotypes, respectively. As for the metabolic efficiency ratio (MER), it was also higher in the muscle tissue lipids of the *CAPN1^{CC}* and *GH^{VV}* genotypes and amounted to 0.41 and 0.42 versus 0.32 and 0.36 units in the peer carriers of the *CAPN1^{GG}* and *GH^{LL}* genotypes.

Table 4: The content of fatty acids in the lipids of the muscle tissue of Kazakh White-headed bulls of different genotypes, %

Name and code of acids	CAPN1			GH		
	CC	CG	GG	VV	LV	LL
Saturated fatty acids						
Caproic (C6:0) + Caprylic (C8:0) + Capric (C10:0)	0.27	0.29	0.27	0.33	0.26	0.44
Lauric (C12:0) + Tridecanoic (C13:0)	1.26	0.95	0.8	1.33	0.87	1.95
Myristic (C14:0)	2.90	2.95	2.01	2.93	3.17	3.14
Pentadecanoic (C15:0)	0.25	0.64	0.95	0.27	0.45	0.33
Palmitic (C16:0)	25.02	24.98	25.19	24.62	25.02	25.64
Heptadecanoic (C17:0)	0.27	0.41	0.44	0.32	0.40	0.54
Stearic acid (C18:0)	17.32	18.02	18.54	17.74	18.14	16.94
Arachidic (C20:0) + Heneicosylic (C21:0) + Behenic (C22:0) + Lignoceric (C24:0)	1.63	1.89	1.91	1.69	1.82	1.9
Monounsaturated fatty acids						
Myristoleic (C14:1) +Palmitoleic (C16:1) +Pentadecylic (C15:1) + Heptadecylic (C17:1)	4.23	3.63	3.61	4.41	3.64	4.29
Oleic (C18:1)	37.95	38.02	39.15	38.15	37.19	37.17
Elaidic (C19:1) + Eicosenic (C20:1 ω ₉) + Erucic (C22:1) + Nervonic (C24:1)	1.65	1.66	1.07	1.59	1.67	1.45
Polyunsaturated fatty acids						
Linoleic (C18:2 ω ₆)	3.44	3.41	3.41	3.50	3.40	3.42
Arachidonic (C20:4)	1.42	1.24	1.10	1.50	1.35	1.23
Linoeladic (C18:2) + γ-Linolenic/ α-Linolenic (C18:3) + Eicosadienic (C20:2) + Eicosatrienic (C20:3 - n ₃ C _{20:4} ω ₆) + Eicosapentaenoic (C20:5 ω ₅) + Docosadienic (C22:2) + Cervonic (C22:6) + Tetracosenic (C23:0)	1.65	1.77	1.55	1.62	1.57	1.64
Σ of saturated	48.92	50.13	50.11	49.23	50.13	50.88
Σ of monounsaturated	43.83	43.31	43.83	44.15	42.5	42.91
Σ of polyunsaturated	6.51	6.42	6.06	6.62	6.32	6.29
LSI	0.97	1.01	1.00	0.97	1.03	1.04
MER	0.41	0.36	0.32	0.42	0.40	0.36

Thus, the results indicate that the muscle tissue of the *CAPN1^{CC}* and *GH^{VV}* genotypes had a higher fat content with a higher proportion of unsaturated

fatty acids, which is desirable in terms of consumer characteristics and dietary properties. In this regard, cattle breeding for beef production

with a low content of saturated fatty acids is considered an opportunity to prevent cardiovascular diseases in humans, which makes it promising to identify genetic markers associated with the fatty acid composition of beef. The DNA typing established the *CAPN1*^{CC/CG/GG} and *GH*^{VV/LV/LL} genotypes for two polymorphic genes among young Kazakh white-headed bulls. *CAPN1*^{CC} and *GH*^{VV} were preferable for increasing meat productivity and quality of meat, including the fatty acid composition of muscle tissue. Carriers of these genotypes had a considerable body weight at 365 days. Their advantage over the *CAPN1*^{GG} and *GH*^{LL} genotypes was 6.9% (P<0.05).

The highest pre-slaughter, slaughter, and carcass weights were obtained from carcasses of *CAPN1*^{CC} and *GH*^{VV} homozygous bulls. Their superiority over the *CAPN1*^{GG} and *GH*^{LL} genotypes was 19.3; 16.2 and 16.4 kg; 19.8; 19.1 and 16.1 kg, respectively. The difference in favor of these genotypes in terms of the meat ratio by 4.1% and 2.0% was provided by a 9.1% greater meat mass and 1.4% and 2.0% slaughter yield. The protein and fat content in the longissimus muscle of the CC and VV genotypes carcasses was higher by 0.28-2.13%. *CAPN1*^{CC} and *GH*^{VV} animals had a higher concentration of unsaturated fatty acids in their muscle lipids than *CAPN1*^{CG}, *CAPN1*^{GG}, and *GH*^{LV}, *GH*^{LL} genotypes, and a higher metabolic efficiency ratio.

Conclusions

In recent years, beef cattle breeding has shown a good pace in Russia. The production of high-quality beef, primarily highly marbled, gives preference to specialized meat breeds, well adapted to the breeding conditions of the South of Russia. These breeds include the Kazakh white-headed bulls. All over the world and in Russia, much attention has recently been paid to genetic markers that control the level of meat productivity and beef quality. Several studies have demonstrated the promise of using the *CAPN1* and *GH* genes. Proteins of the calpain-calpastatin system have been proven to participate actively in the post-slaughter decomposition of muscle.

Horses of the Aberdeen-Angus, Hereford, Limousine breeds, and their crosses in different breed combinations, homozygous or

heterozygous for the C allele in the *CAPN1* gene, have been found to have tender meat, less resistance to cutting, and a 14-20% greater marbling "Compared to homozygous GG animals. It was reported that the relationship between genotypes in the *CAPN1* gene with the level of meat marbling and a change in its color after six days of maturation [39].

The frequency of the desired C allele is observed in the Japanese Wagyu breed - 0.76 and Aberdeen Angus - 0.71 [43-45]. Our studies revealed a significantly lower frequency of occurrence that amounted to only 0.12, indicating the need for animal genotyping and targeted selection of parental pairs to obtain offspring with C allele. The obtained data on the superiority of *CAPN1*^{CC} genotypes in live weight, indicators of carcasses during slaughter, and chemical and fatty acid composition justify the feasibility of such an approach.

Another gene associated with productivity traits that have been shown in several works is the *GH* gene, or somatotropin, an endogenous factor with lactogenic, insulin-like, fat-mobilizing, and neurotropic effects [46, 47]. It was found that the average weight of *GH*^{AA} and *GH*^{AB} Japanese Wagyu bulls was significantly higher than that of *GH*^B bulls. In addition, the level of growth hormone was also higher in the blood plasma of these animals [48, 49]. It was demonstrated that the *GH* allelic polymorphism affects the thickness of the fat layer between the fibers of muscle tissue in Wagyu animals and their crosses with the Limousine breed [50-53].

It was reported that bulls carrying the A allele in the *GH* gene had a greater thickness of ribs and a lower melting point of fat. Animals with B and C alleles had a higher content of C_{18:1}. In contrast, the C allele was associated with high monounsaturated and unsaturated fatty acids. In heifers, in addition to these regularities, the presence of the A allele had a significant positive effect on carcass weight, loin eye area, rib, and subcutaneous fat thickness, while the B allele influenced loin eye area and rib thickness [53].

Our results also indicate the influence of the allelic state of the *GH* gene on the meat parameters of the Kazakh white-headed bulls. Despite a higher fat

content in the longest dorsal muscle of the *GH^{vv}* bulls, however, it had a higher amount of unsaturated fatty acids and the most desirable dietary properties of meat - oleic (C_{18:1}) and linoleic (C_{18:2}) acids. The best ratio of unsaturated to saturated fatty acids and the arachidonic (C_{20:4}) and linoleic (C_{18:2}) acids determined the best lipid saturation indices and metabolic efficiency indices. SNPs associated with the fatty acid composition of beef are found in the genes of stearoyl-CoA desaturase and fatty acid desaturase (*SCD*, *FADS2*). They are considered promising markers for breeding Japanese beef cattle [54, 55]. The accumulation of data on the influence of the *GH* and *CAPN1* genes on the meat productivity of cattle of different breeds will make it possible to select animals with the desired genotypes more accurately and efficiently to get high-quality beef.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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